

ON SOME PELAGIC DOLIOLID TUNICATES (THALIACEA, DOLIOLIDIDA) COLLECTED BY A SUBMERSIBLE OFF THE EASTERN NORTHAMERICAN COAST

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ABSTRACT

Specimens of Doliolids collected from a submersible at several stations off the eastern coast of North America were examined. Four species were identified, of which three were described by Godeaux (1996). Of these, one belongs to the new genus, *Paradoliopsis* (Godeaux, 1996). It is proposed that the order Doliolidida be divided into two suborders: the Doliolidina (animals with eight muscle bands), and the Doliopsidina (animals with five muscle bands). Each suborder is represented in our collection by two families. For the Doliolidina these families are the Doliolidae (*Doliolinetta intermedia*) and the Doliopsoididae (*Doliopsoides atlanticum*), and for the Doliopsidina the families are the Doliopsidae (*Doliopsis bahamensis*) and the Paradoliopsidae (*Paradoliopsis harbisoni*).

The family Doliolidae is the best known group of the tunicate order Doliolidida. The vertical distribution of members of this family has been well documented with the use of multiple opening and closing nets. The various stages of the complex life cycle of the different species of Doliolidae are located in the epipelagic and mesopelagic layers. They are mainly found at depths between 50–100 m, where they graze on small autotrophic algae (Weikert and Godeaux, unpubl.). Doliolids are so fragile that they are easily damaged, making their identification difficult. Identification and determination of the various stages in the life cycle is made even more difficult by the fact that several different species are often mixed together in a single net collection. In intact doliolid colonies, the dorsal spur is many times longer than the nurse, yet it is always missing in net-collected specimens. Species of doliolids are rarely collected below 500 m and usually only non-feeding nurses are found.

These difficulties are circumvented by the use of in situ SCUBA-based techniques of collection, which enable researchers to collect individual colonies up to a depth of 30 m. Thus nurses, together with the zooids attached to their dorsal spur, can be photographed, collected and preserved as discrete samples. Using this technique, Tebeau and Madin (1994) were able to photograph and collect nurses of *Dolioletta gegenbauri* with a dorsal spur to which trophozooids and phorozooids were attached.

The use of manned submersibles with appropriate collecting devices (Youngbluth, 1984) has allowed in situ collections to be made at greater depths. As a result, a number of new genera and species of delicate gelatinous zooplankton such as medusae (e.g., Larson et al., 1988; Tuesen, 1993), siphonophores (e.g., Pugh, 1992, 1995), ctenophores (e.g., Madin and Harbison, 1978) and appendicularians (e.g., Fenaux and Youngbluth, 1990, 1991) have been described.

During several oceanographic cruises between May 1983–November 1989, dives were made by one of us (G.R.H.) with the JOHNSON-SEA-LINK submersibles in which various doliolids were collected (Table 1). Specimens were collected between 500–800 m depth off the eastern North American coast (George's Bank, the Bahamas, and the Dry Tortugas, Table 2).

Table 1. Stations where specimens of Doliodiids were collected with the JONHSON-SEA-LINK submersibles. Station Number is composed of the name of the submersible (JONHSON-SEA-LINK I or II) and its dive number. Collections were made in three locations, which are indicated in front of the Station number: a) Bahamas, b) Dry Tortugas, c) George's Bank. Dive times are local times.

Station number	Location	Dive time	Date
a) JSL II 0956	23°07'N, 78°43'W	0624-0835	16 Oct 1984
a) JSL II 0970	26°33'N, 78°03'W	0030-0230	21 Oct 1984
a) JSL II 0981	25°54'N, 77°18'W	1900-2130	24 Oct 1984
a) JSL II 0985	25°03'N, 77°32'W	0230-0500	27 Oct 1984
a) JSL II 0988	25°23'N, 77°56'W	0330-0540	28 Oct 1984
a) JSL II 0991	25°23'N, 77°58'W	1900-2130	29 Oct 1984
a) JSL II 0995	25°24'N, 77°58'W	2330-0130	30 Oct 1984
a) JSL II 0997	25°23'N, 77°55'W	1900-2130	31 Oct 1984
a) JSL II 0998	25°22'N, 77°50'W	2330-0130	31 Oct 1984
a) JSL II 1004	25°22'N, 77°50'W	2330-0130	02 Nov 1984
b) JSL II 1454	24°31'N, 83°45'W	1538-1800	30 Aug 1987
b) JSL II 1458	24°01'N, 82°17'W	1520-1820	03 Sep 1987
a) JSL II 1679	25°50'N, 77°15'W	1227-1605	07 Oct 1988
b) JSL II 1681	26°25'N, 77°53'W	1218-1558	08 Oct 1988
c) JSL I 2145	40°02'N, 69°02'W	1245-1615	06 Aug 1987
a) JSL I 2660	26°23'N, 77°48'W	1225-1600	19 Nov 1989

Examination of this material has allowed us to identify species belonging to four different genera. Investigation of this material was facilitated by comparison of the color photographs of the living animals (which showed patterns of pigmentation) with the corresponding preserved individuals.

MATERIAL AND METHODS

Specimens of doliodiids were collected using the detritus samplers on the JOHNSON-SEA-LINK submersibles (Youngbluth, 1984). These sampling devices allow individual colonies to be collected, which eliminates uncertainty as to whether or not particular zooids belong together, a problem with most other collection techniques. After collection, specimens were maintained on board ship at 5°C, which approximated the temperatures at which they were collected. The specimens were photographed shortly after collection while they were still alive with a Zeiss Tessovar macrocamera. They were then preserved in a 3% glutaraldehyde-seawater solution.

The preserved animals were stained with chlorantine red and examined in glycerin with a Zeiss Opton microscope and a Zeiss photomicroscope. A few specimens were also examined with an electron microscope. The removed organs were postfixed in 1% OsO₄ and embedded in epoxy resin (glycidether 100, Serval) using standard procedures. Ultrathin sections were cut with a Reichert Jung (Ultracut E) ultramicrotome fitted with a diamond knife. They were contrasted with uranyl acetate and lead citrate before observation with a Jeol 100 SX transmission electron microscope at 80kV accelerating voltage.

Table 2. Specimens collected: Specimen number consists of the dive number and the number of the sampling device. (1) Indicates that the specimen was identified from photographs alone, and (2) indicates that developing buds were present. Ambient temperature was measured and recorded while the specimen was collected.

Specimen number	Species	Collection depth (m)	Temperature (°C)
0956-04	<i>Doliopsis bahamensis</i> (1)	625	12.7
0970-02	<i>Doliopsis bahamensis</i>	573	13.5
0981-06	<i>Doliopsis bahamensis</i>	643	12.3
0985-01	<i>Doliopsis bahamensis</i>	518	13.0
0991-05	<i>Doliopsis bahamensis</i>	579	12.6
0995-02	<i>Doliopsis bahamensis</i>	549	12.1
0997-07	<i>Doliopsis bahamensis</i>	695	11.8
0998-07	<i>Doliopsis bahamensis</i>	582	12.1
1004-08	<i>Doliopsis bahamensis</i>	598	11.4
1458-01	<i>Doliopsis bahamensis</i> (2)	719	5.6
1679-01	<i>Doliopsis bahamensis</i> (2)	755	9.8
1681-02	<i>Doliopsis bahamensis</i> (1)	814	8.8
2145-08a	<i>Doliopsis bahamensis</i>	739	4.8
2660-01	<i>Doliopsis bahamensis</i> (2)	548	13.3
1458-02	<i>Doliolinetta intermedia</i>	746	5.5
0981-04	<i>Doliopsoides atlanticum</i>	607	13.0
0988-07	<i>Doliopsoides atlanticum</i>	671	10.9
1004-07	<i>Doliopsoides atlanticum</i>	648	10.7
1454-07	<i>Paradoliopsis harbisoni</i> (2)	735	5.7
2145-08b	<i>Paradoliopsis harbisoni</i> (2)	739	4.8

OBSERVATIONS

Doliolinetta intermedia (Neumann, 1906)

The colony 1458-02 (Table 2, collected near the Dry Tortugas) contained a typical doliolid nurse, several dozen trophozooids and two phorozooid buds.

Nurse. (Figs. 1,6A).—The elongated barrel-shaped nurse (2.5 cm along the major axis, the posterodorsal spur not included, diameter 1.5 cm) has nine narrow muscular hoops (I–IX); the first ring is the buccal sphincter, the last is the atrial sphincter. The siphons are at opposite ends of the animal. Only muscle VII is open dorsally, with its free branches running backwards to the dorsal spur. This process bears several large trophozooid buds that are visible in the photographs. The body is covered with a thin layer of tunic (Figs. 1A,6A).

As usual, the anatomy of the nurse is very simple. Most of the internal organs (endostyle, peripharyngeal bands and spiral organ, digestive tract, and branchial septum) are not present. The only organs remaining are the nine muscle hoops, the dorsal neural ganglion (brain) with its nerves, the ventral cardiopericardium and attached stolon.

The buccal siphon has ten rounded flaps, and the atrial siphon has five ventral finger-like papillae, each of which is provided with a basal bulb. The papillae are separated by folds of tunic.

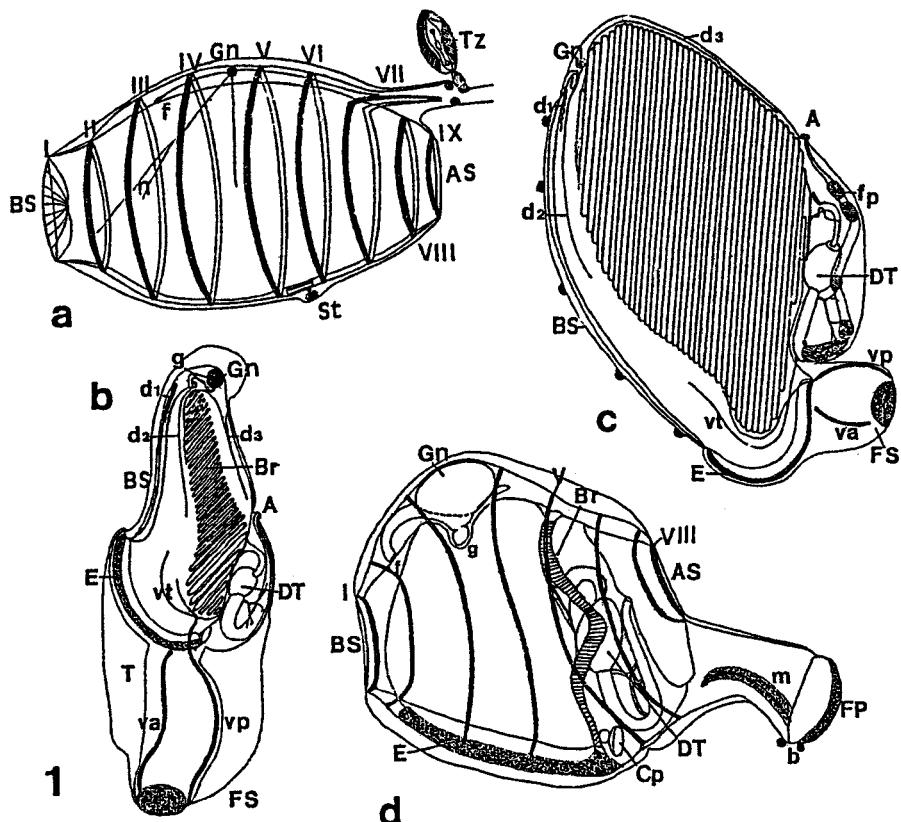


Figure 1. *Doliolinetta intermedia*. A) Lateral view of the nurse bearing a trophozooid bud on the dorsal spur; B) Lateral view of a young trophozooid bud; C) Full-grown trophozooid; D) Developing phorozoid bud with two gonozoid buds on the ventral process.

The muscles are slender (at most 500 μm wide). They seem to be formed of two distinct, equal, and parallel-running elements, which sometimes show fibrils. Each intermuscular space is 2.5 mm wide. The nurse is therefore stenomyonic aclinous (Garstang, 1933).

The neural ganglion (brain) is rounded and is located in front of muscle V, three quarters of the distance between muscles IV and V. Several nerves radiate from the brain: a median anterior nerve, a single pair of antero-lateral nerves, a pair of lateral nerves and a pair of posterior nerves passing over muscle V. The unpaired anterior nerve reaches muscle I, while the antero-lateral nerves divide and run obliquely to muscles II and III, and possibly on to I and IV. The lateral nerves supply muscle V. Every posterior nerve

FIGURE ABBREVIATIONS. I-IX: muscle rings; A: anus; AS: atrial siphon; b: gonozoid buds; Br: branchial septum; BS: buccal siphon; c: stomach caeca; Cp: cardiopericardium; DT: digestive tract; d 1,2,3: dorsal muscles of the trophozooid; E: endostyle; f: ciliated funnel; fp: fecal pellets; FP: fixation plate; FS: fixation stalk; G: gonads; g: neural gland; Gn: neural ganglion (brain); lm: lateral muscle; m: muscles of the stalk; n: nerves; Oe: esophagus; Ov: ovary (+ oocyte); p: white pigment clusters; Pb: peribranchial bands; PG: pyloric gland; S: stomach; St: stolon; T: tunic; Tz: testis; Tz: trophozooid bud; va: ventro-anterior muscle; Vm: ventral muscle; Vo: vibratile spiral organ; vp: ventro-posterior muscle; vt: ventro-transverse muscle.

divides in front of muscle VII: one branch runs along the free end of the muscle, another can be followed to muscles VIII and IX. The ganglion also produces a thin anterior cellular thread, which ends at the level of the trumpet-shaped ciliated funnel (80- μ m long), which opens into the pharyngeal cavity slightly behind muscle III. The thread and funnel are the sole remnants of the former larval neural tube. The neural cavity also contains a transient neural gland, which is always absent in full-grown animals (Godeaux, 1957-1958, pp.185-216).

On the left side, the remnant of the statocyst is visible in front of muscle III. In this species, the statocyst is an ectodermal cup covered by the tunic; the statolith is lost, as is usual in these doliolid nurses (e.g., *Doliolina muelleri*, Godeaux, 1957-1958, fig. 90).

The ventral cardiopericardium is conspicuous between muscles V and VI. It has the classical tubular structure of the tunicate heart; its transverse musculature is partly masked by an epithelium, which corresponds to the former supraccardial vesicle characteristic of the Doliolidae as described by Godeaux (1957-1958, figs. 81,86). The elongate stolon protrudes below the rear of the cardiopericardium and is faced with a local ectodermal thickening where numerous phorocytes are observed.

The dorsal process of this specimen is incomplete (compared with the Figs. 6A,B,C), and most of the buds and delicate trophozoids were released and damaged after preservation.

Trophozoid.—Two young trophozoid buds (2- and <3-mm high respectively) made it easier to interpret the structure of the full-grown animal (Figs. 1B,5A,6B,C). The spoon-shaped body is covered with a thin layer of tunic. The upper part of the anterior face is occupied by the buccal aperture, an elongate opening devoid of a sphincter, but supplied with five pairs of (sensory?) papillae. The buccal aperture is outlined by the two peripharyngeal bands arising from the curved endostyle which lies just below it. The ventral wall contains a long attachment stalk (1/3 of the total height of the bud), which joins the phorozoid to the nurse spur. Attachment to the spur is through a distally rounded and thickened ectodermal plate, which is devoid of tunic. This plate may function as a kind of placenta.

The neural complex lies at the top of the animal: the neural gland is still present and corresponds to the lumen of the larval neural tube. This tube opens into the pharyngeal cavity through the ciliated funnel which is also tied to the left peripharyngeal band. The peripharyngeal bands unite behind the ganglion forming a volute, the apical spiral organ. The musculature of buds is very weakly developed and only a few muscles can be observed easily. The muscle pattern is more visible in full-grown trophozoids.

The pharyngeal cavity is spacious. The branchial septum (with a single row of horizontal slits on both sides of the digestive loop) forms the rear limit of the pharyngeal cavity. There is no atrial cavity, which disappears during embryogenesis, and the anus directly opens to the exterior.

Fully developed trophozoids are 5-8 mm high, including the 1.5 mm long stalk (Figs.1C,6C). Fecal pellets in the intestine demonstrate that they are active filter feeders. Their structure, when compared to that of the buds, shows changes in the relative positions of different organs due to allometries of growth.

The buccal siphon occupies the entire anterior face of the animal. The ventral endostyle extends the whole length of the pharynx, having pushed the globular attachment stalk to below the digestive tract. The endostyle is a rather deep groove; its cross section reveals a median ciliary band and on both sides a pair of superposed lateral bands, the

lower with a few large glandular cells, the upper with ciliary cells. The superior part of the endostyle is a thin veil, which bears an apical band of ciliary cells at the limit of the pharyngeal epithelium. The endostyle gives off the anterior ciliary peripharyngeal bands encircling the buccal opening, and the posterior ciliary raphe joining the esophagus; its hinder part lies at the level of the bend of the intestine.

The neural ganglion (brain) displays two anterior nerves on both sides of the thread ending at the ciliary funnel, a single lateral nerve and a single thick posterior nerve.

The weakly developed musculature consists of two groups of muscles; one dorsal, the other ventral. The dorsal group is composed of three muscles. A very thin muscle makes a hoop above the buccal aperture and resembles the upper part of an incomplete buccal sphincter. A weak second muscle is seen on both sides of the animal behind the peripharyngeal band. The symmetrical elements of this muscle fuse in front of the neural ganglion. The broad third muscles are visible dorsally between the two series of gill slits. They begin at the upper anterior margin of the branchial septum and pass on both sides of the ganglion. Dorsally, both muscles run parallel to one another before fusing into a single median component ending above the anus.

The ventral group is also composed of three muscles. A transverse muscle runs in front of the digestive tract, below the gills, and corresponds to the second dorsal muscle. Following Grobben (1882), one may imagine that these muscles represent a former circle of muscle as is present in the other forms of dolioiids. The two other ventral muscles make up the muscles of the fixation stalk and both extend from the fixation plate. The anterior muscle ends just below the endostyle; the posterior muscle crosses the endostyle on one side (thus asymmetrically) and extends into the cavity of the body; it also crosses the transverse muscle but does not fuse with it. The ventral muscles and the third dorsal muscles are composed of two broad parallel fibers.

Both sides of the branchial wall have a single series of some 35–50 narrow transverse slits with densely packed long flagellae, depending on the size of the specimen. Apparently these slits result from the progressive division of a single embryonic protostigma.

The digestive tract is U-shaped (Figs. 1C, 5A). A white pigment on its middle part appears to be present. The curved esophagus opens at the mid-height of the pharynx and bears an obvious spiral thickening; it opens into a globular stomach, which is followed by a long intestine. The pyloric gland emerges from the duodenum; it soon divides into two main branches, the first of which is turned towards the bend of the intestine, and the second of which runs towards the mid-height of the ascending limb, where it again divides. This gland is not forked as in *Doliolina muelleri* (Godeaux, 1954), but forms a network of small tubules running along the main axis of the ascending intestine. The anus opens *above* the level of the esophageal funnel. The gill slits directly open to the exterior, since as the atrial cavity is missing.

The short fixation stalk is a haemocoelian diverticulum limited by a thin ectodermal epithelium and protected by a thick layer of tunic (less developed elsewhere), which is absent at the level of the distal fixation plate.

Phorozoid.—Besides the trophozoids, two phorozoid buds were present (Fig. 1D). These individuals are of great utility for precise identification to species (cryptic species, Godeaux, 1961). Their stage of development allows a comparison with similarly developed buds of *Doliolina muelleri* (Godeaux, 1957–1958, p. 102).

The sizes of these buds are respectively 630 μm and 660 μm for the trunk (plus, for the largest animal, a 160 μm -long fixation stalk and a maximum height of 600 μm). The different organs, although still embryonic (cubic epithelia), are clearly identifiable.

The siphons are open in the large specimen and the tunic is missing around them. Possibly, the individual was not yet feeding since no fecal pellets are present in the intestine. Eight thin muscles are visible.

The neural complex is composed of a dorsal dense ganglion and a ventral and obviously hollow neural gland, which opens (at the site of the future ciliary funnel) into the pharynx, in front of muscle III. The transient aneural strand is conspicuous behind the ganglion and neural gland.

Ventrally the long endostyle begins in front of muscle II and approaches muscle V, but the individual is too small to allow a more precise determination. Interestingly, the branchial wall is shaped like a vertical circumflex accent at the level of muscle V. Numerous stigmata are already open (25 in the large specimen). The digestive tract is U-shaped, which means that the specimens belong to the genus *Doliolina* (Garstang, 1933). Between the two limbs, the pyloric gland divides into three main tubes, ramifying along the ascending intestine.

The ventro-posterior fixation stalk projects from between muscles VI and VII. Two symmetrical longitudinal muscles run along its axis. Two gonozoid buds can be discerned on its distal end.

Discussion.—Recently Godeaux (1998c) proposed that the genus *Doliolina* can be divided into two subgenera, namely *Doliolina* and *Doliolinetta*, based on differences between the shape and position of the gonads in gonozoids. In the subgenus *Doliolina* Borgert, 1894, the testis is massive and lies close to the intestinal tract, as in *Doliolina muelleri*, while in the subgenus *Doliolinetta* Godeaux, 1998c, the testis is stretched along the left side of the animal and reaches muscle II, as in *Doliolina* (now *Doliolinetta*) *intermedia*. The combined analysis of the three stages in the present collection simplifies a precise identification to species.

The nurse (old oozooid) is remarkable for its large size, only known until now for the genus *Dolioletta*. It differs from the large nurses of *Dolioletta* sp. and of *Doliolum* sp. in the extreme slenderness of its muscles (stenomysionic aclinous nurse), the width of the intermuscular spaces, the presence of a single anterior pair of nerves, and the absence of a statocyst. Nurses of this type are only known in the subgenus *Doliolinetta* (Godeaux, 1998c) in the species *Doliolinetta indicum*, *Doliolinetta resistibilis* and *Doliolinetta intermedia*. These nurses differ from those of *Doliolina muelleri* and *Doliolina krohni* which are characterized by wide muscles separated by narrow gaps (eurymyonic amphiclinous nurse, Garstang, 1933). Garstang (1933) identified a large nurse (15 mm long) provided with thin muscle bands, and caught in the southern Pacific Ocean (vertical haul 0–500 m), as *Doliolina intermedium* (Neumann 1906), now *Doliolinetta intermedia* after Godeaux (1998c). Thus, our specimen belongs to the subgenus *Doliolinetta*.

The trophozoooid is described here for the first time. This large zooid is distinguished by the great number (from 35–50) of gill slits (a number [up to 40] observed only in *Dolioletta gegenbauri*, Braconnat, 1970), by the spiral along the esophagus, and by the relative position of the anal aperture *above* the level of the esophageal funnel (differing from the description of the small trophozoooid of *Doliolina muelleri* given by Braconnat, 1970; see also Godeaux, 1998b).

The phorozooid has a vertical U-shaped digestive tract, a very long endostyle beginning in front of muscle II, a vertical branchial septum close to muscle V, which has numerous slits. These characters are found in the species *Doliolinetta intermedia* (Neumann, 1913a,b), and are in agreement with a recent figure of the gonozooid of *Doliolinetta intermedia* (Godeaux, 1998c).

Garstang (1933) claimed that the species *Doliolina* (now *Doliolinetta*) *resistibile* and *Doliolina intermedium* (now *Doliolinetta intermedia*) are so closely related that *Doliolinetta resistibile* is merely a variant of *Doliolinetta intermedia*. His hypothesis is not supported by Neumann's description of the two species (based on the length of endostyle and the location of the branchial septum, Neumann, 1913a,b). Furthermore, *Doliolinetta intermedia* is a northern species while the southern *Doliolinetta resistibile* occurs close to the Antarctic ice pack (Neumann, 1913b; O'Sullivan, 1983). Based on the characters found in the three stages that were collected, the preponderance of evidence suggests that this sample belongs to the species *Doliolinetta intermedia*.

Doliopsoides atlanticum Godeaux, 1996

An illustration of this species was erroneously labeled *Doliopsoides bahamensis* by Godeaux (1998a, figs. 17,13a).

Three barrel-shaped specimens of this species (Fig. 2,6D) were collected in the Bahamas (Table 2). The sizes of these specimens are A (Station 981) l = 8 mm, h = 7.7 mm; B (station 988) l = 7.5 mm, h = 6.5 mm; and C (station 1004): l = 6.5 mm, h = 5.2 mm.

The tunic is very thin over the entire organism, and is sometimes covered with foreign material (which masked the internal structure of specimen C). The underlying ectoderm is also very thin. The possible presence of pigmented areas could not be determined since all photographs that were taken were black and white.

The body is ovoid with the siphons occupying the ends of the main axis. The anterior buccal siphon is 2 mm wide and has 18–20 rounded and contiguous flaps in front of the pharynx aperture.

The animal is provided with eight narrow muscle bands as in the blastozooids of *Doliolum*, but the muscles are arranged in a different pattern. Most muscles form complete rings. The number of fibers is low, the widest muscles being II (10 fibers), III (12–14 fibers) and VII (8 fibers). Some intermuscular gaps are wide (II–III, III–IV and IV–V).

Muscle I is immediately behind the buccal siphon. Muscle II, which also forms a complete ring, is close to muscle I. A true buccal vestibule is lacking.

Muscle III is an 85 μm -wide ring, passing *behind* the neural ganglion (brain), a feature that differentiates this genus from *Doliolum*.

Muscle IV also forms a complete hoop, but bears a sigmoid lateral branch linking it to muscle V. This transverse junction is made of two fibers that pass from one muscle to the other; this junction is characteristic of the genus.

Muscles V and VI are different, and their configuration is the main distinguishing characteristic of the genus. Muscle V is continuous ventrally, but is open dorsally and each branch fuses with the corresponding dorsal free extremity of muscle VI: a muscular arch is therefore present on both sides of the animal. Further, muscle VI is open ventrally, with its branches first extending forwards then backwards, finally ending below the atrial siphon. Only a study of embryogenesis could determine whether muscles V and VI constitute a single muscle or whether they are indeed separate muscles.

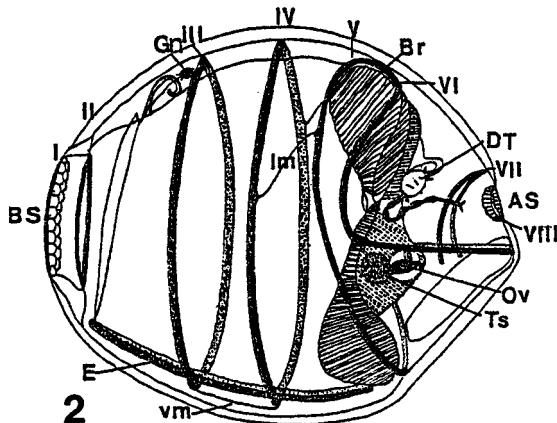


Figure 2. *Doliopsoides atlanticum*. Ventral view of a full-grown gonozoid.

Muscle VII is also open ventrally; its free ventral extremities extend forwards and pass a short distance *beneath* the branches of muscle VI.

Muscle VIII is the narrow atrial sphincter.

Further, a thin medio-ventral muscle (2 fibers wide) running below the endostyle extends between muscles III-IV, which exchange fibers. This muscle is also specific to the genus.

A rounded neural ganglion (brain) lies dorsally between the volute of the peripharyngeal bands and muscle III. Several nerves emerge from it: a median and thin longitudinal nerve which supplies muscle I, two anterior nerves obliquely running towards muscles I and II, and two large posterior nerves passing *over* muscle III (as in *Doliolum*) before dividing towards the rear. No lateral nerves were observed. There is no trace of a statocyst. An antero-median cellular thread links the ganglion to a ciliated funnel, which is tied to the locally broadened left peripharyngeal band. The neural gland is absent but, as shown by Godeaux in *Doliolum* (1957-1958), the ciliated funnel and cellular thread indicate the former existence of this early-disappearing organ (which is present in colonial ascidians and in *Pyrosoma*, Godeaux, 1956-1957, 1957-1958; Godeaux and Debroux, 1979).

Two slender threads, with a distally free end, were observed extending laterally and slightly beneath the ganglion only in specimen B. These structures could be the remnants of a former vascular system linking neighboring blastozoids on a stolon. However, these structures were completely missing in the two other specimens. No trace of a stolon was found.

The endostyle is long and slender. It begins slightly behind muscle II and ends in front of muscle V at the level of the branchial septum. As usual, the two peripharyngeal bands arise from its anterior extremity, run parallel to muscle II and dorsally form the spiral organ lying in front of the ganglion but behind the ciliary funnel. The retropharyngeal raphe is a thin thread sliding between the two rows of branchial slits. A hyaline vesicle that is visible in specimens A and B could be the pericardium. This vesicle is located at the rear of the endostyle close to the origin of the retropharyngeal raphe, where the pericardium is located in other doliolids. Foreign material hindered the examination of this structure in specimen C.

The branchial basket is slightly tilted. It begins at the level of the arches of muscles V and VI, and rests between the endostyle and muscle V. A single series of transverse slits is present on both sides; each is twisted at the level of the esophagus with 20 dorsal and 30 ventral slits.

The digestive tract is an enlarged U-shaped loop, inclined in the sagittal plane (Fig. 5B). The narrow esophagus opens into the pharynx through a funnel and forms a vertical semicircle. It gives off a longitudinal whorl, which spirals down to the stomach. The elongate, pouch-like stomach has numerous caeca scattered on its lateral walls, particularly in the vicinity of the openings of both the esophagus and duodenum. The duodenum is smooth and thin. It gives off the pyloric gland, which is a tube that is more or less complex in different individuals. This gland consists of a single long canal closely applied against the ascending limb of the intestine in specimen A. However, the main tube divides into three branches, each of which further divides into tubules running along the ascending intestine, in specimens B and C. Glands of this type are never observed in species of *Doliolum*. The wall of the intestine is covered with small papillae. Fecal pellets are present. The anus is located at the mid-level of the stomach; it has a smooth rim, and opens into the small atrial cavity at the level of muscle VII but below the atrial siphon. This aperture is encircled by muscle VIII and is provided with some twenty elongate lappets, with an ectodermal base and a layer of tunic.

The gonads of the three specimens (Fig. 2) are located immediately below the digestive loop and behind the branchial basket. The relative stages of development are different in each of the three specimens.

In animal A, the testis appears as a poorly-defined, two-lobed mass ($830 \times 500 \mu\text{m}$), transversally located in front of a cavity which contains the ovary and a small oocyte (diameter = $170 \mu\text{m}$). The vas deferens emerges from the hilum and runs downward on the left side of the ovary. Spermiduct and oviduct unite behind the genital complex and discharge into the atrial cavity. In animal B, the genital mass is composed of an anterior two-lobed testis ($850 \times 470 \mu\text{m}$) and a small ovary with a voluminous oocyte (diameter = $500 \mu\text{m}$). In animal C (Fig. 6D), the smallest of the three, a large oocyte (diameter = $750 \mu\text{m}$) is seen on the left of the sagittal plane beside the testis ($480 \times 430 \mu\text{m}$).

The species *Doliopsoides atlanticum* is hermaphroditic and possibly protogynous.

The relative positions of the digestive tract and the gonads are different from their position in *Doliopsoides horizonti* Tokioka and Berner, 1958 (Fig. 5C).

Discussion.—Taken together, the characters presented by the three specimens justify their attribution to the genus *Doliopsoides* Krüger 1939. Only a few individuals, mainly gonozooids, have been described until now. Krüger described 12 gonozooids from 10 stations in the Atlantic Ocean, mostly collected below 400 m, between latitudes 25° and 45°S (type species *Doliopsoides meteori* Krüger, 1939). Later, Tokioka and Berner (1958 a,b) studied several gonozooids and the only known phorozoid, caught at two stations off the Peruvian coast (between 8° – 10°S), which they named *Doliopsoides horizonti*. The phorozoid has a short ventral protuberance into which the free ends of muscle VII project. An isolated 5-mm long gonozooid has also been recorded from the Indian Ocean (38°S) and described by Godeaux and Meurice (1978).

Nothing is known of the life cycle. Most probably, development occurs at depth. The existence of several successive stages must be surmised (similarly to *Doliolum*) if indeed the only phorozoid that has been observed is not simply an immature gonozooid. Since a stolon has never been observed in specimens, the existence of a blastogenetic

oozoid can be hypothesized. Otherwise the life cycle could be limited to only a single generation.

Although the genus is well defined, the species differ in only minor details of their anatomy, possibly explainable by the fragility of the animals (most of them were caught at depth) and by damage after preservation, due to the weakness of their musculature.

Our specimens are similar to those described by Tokioka and Berner in that they have the small ventral muscle extending between III-IV and not between II-III as reported by Krüger. They differ in their larger dimensions, the lobes of the buccal siphon, and the less inclined gut, but mainly in the configuration of the genital mass, in which the testis lies in front of the ovary (Figs. 2,5C).

Species of *Doliopsoides* closely resemble species of *Doliolum*. They are differentiated mainly by the pattern of the musculature. However, the relative positions of most organs (endostyle, spiral organ in front of the neural ganglion, digestive loop, branchial basket, gonads), are identical to those of the corresponding organs in species of *Doliolum* (Godeaux, 1996).

Doliopsis bahamensis Godeaux, 1996

This species (Figs. 3,6E,F) was collected at 14 different stations, all but three of them in the Bahamas (Tables 1,2). All of them appear to belong to a species of the genus *Doliopsis* (*Anchinia*), and the collection contains more or less well-developed phorozoids and gonozoids. The structure of the younger specimens facilitated the interpretation of the anatomy of the very more fragile adults, which were often in poor condition.

The two very young individuals used for description were selected from the collection made at Station 2660 (Tables 1,2).

The first bud (Fig. 3A) is 3 mm long between the siphons and 3 mm high, not including tunic (4 mm high when the tunic is included). The tunic is thicker on the dorsal side and immediately below the endostyle. Numerous cells are scattered throughout the matrix of the tunic. The underlying ectoderm is thin.

The siphons are open, although they are still covered by a thin layer of tunic. The buccal siphon is provided with eighteen rounded adjacent flaps; the atrial siphon has twenty-two low, elongated and pointed projections. The buccal siphon appears less open than the atrial aperture.

The five muscles are slender but well outlined. Muscle hoops I and II are closely associated with the buccal aperture. On both sides of the pharyngeal wall, the rather short muscle III appears in the shape of a sigma. Muscles IV and V, located behind the branchial septum, are complete rings, forming the atrial sphincter.

The neural system (brain) still displays embryonic characters. A remnant of the neural gland can be seen below the dense ganglion; it opens forwards into the pharynx through the neural canal and the future ciliary funnel. An aneural strand is still present behind the ganglion.

The peripharyngeal bands run behind muscle II and unite *behind* the ganglion, forming a spiral vibratile organ occupying the *top* of the animal. The anlage of the future ciliary funnel is linked with the left band.

The straight endostyle groove lines the ventral side. In front, it gives off the two peripharyngeal bands and in the rear, it gives off the retropharyngeal raphe. The cardiopericardium can be seen below the digestive tract close to the rear of the endostyle (Fig. 3).

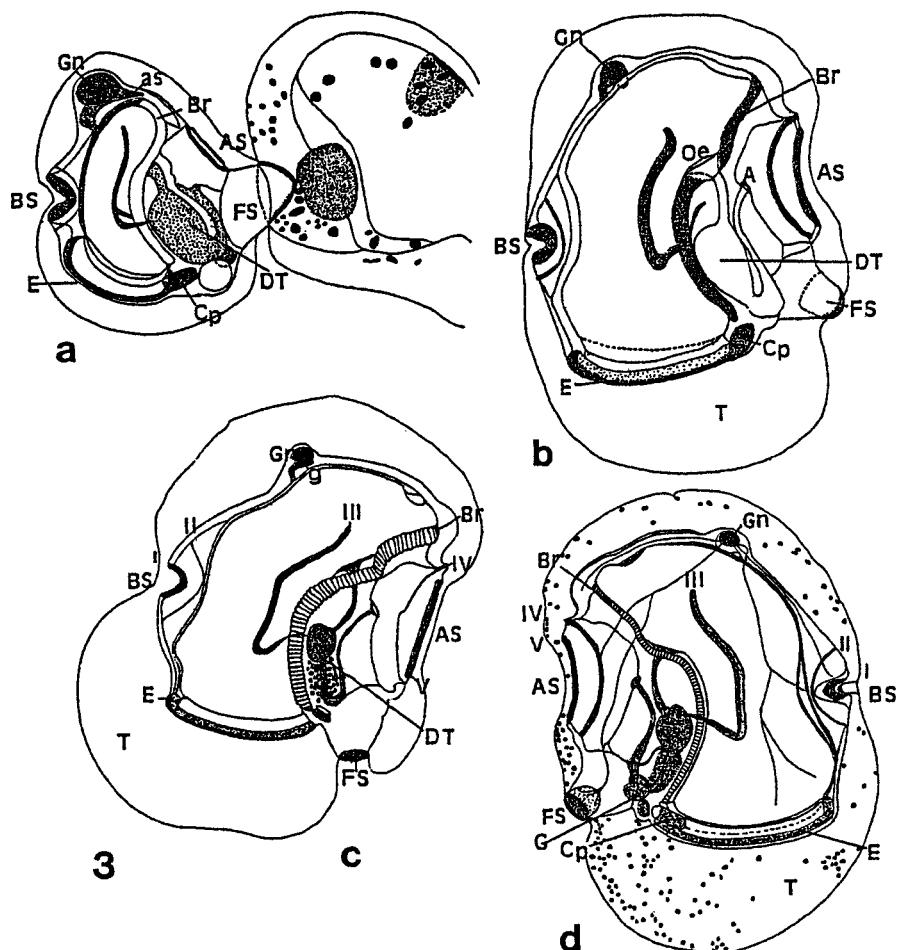


Figure 3. *Doliopsis bahamensis*. A) Developing bud attached to the ventral process of the carrier; B,C) Developed phorozoid buds (no gonadal anlagen); D) Full-grown but still immature gonozoid.

The S-shaped branchial basket is divided in two parts united at the level of the esophagus. Fifty slits were counted, of which thirty are ventral.

The digestive tract is U-shaped, elongated in the sagittal plane. The esophagus is thin and rather long and is followed by a globular stomach opening into the descending limb of the intestine; this part is concealed by a thick cover of mesenchymatous cells (pigment cells or nephrocytes perhaps). The ascending limb is thin, naked and devoid of fecal pellets since the animal was not yet feeding (the buccal siphon is covered with a thin layer of tunic). The anus lies above the level of the esophageal funnel. The fixation stalk is ventro-posterior and entirely embedded into the tunic; only a naked button-like fixation plate still protrudes.

A second young individual confirms these observations (Fig. 3B). Its length between the siphons is 1.5 mm and the height without the tunic is 1.5 mm. The tunic is very thick below the endostyle, and at the level of the neural ganglion. The whole animal is embedded in a spherical capsule. Numerous cells are scattered in the matrix of the tunic.

The neural gland and its canal are conspicuous below the ganglion. Their appearance is quite similar to that displayed by a *Doliolum* bud. The branchial system already has open slits, but the compactness of the body prevents counting. The gut is empty. Feeding seems to be commenced at a size above 5 mm.

Full-grown specimens are very fragile (Figs. 3C,D,6E,F). The viscera appear concentrated in the rear of the body. Moreover, the relative proportions of different parts of the animal differ somewhat from those of the buds. The animals chosen for description are from the collection made at station 995 (Tables 1 and 2).

The length between the siphons reaches 9–10 mm, and the height at the level of the apical spiral organ is ca 10 mm. Thus the animals are slightly shorter than tall. The tunic is sticky, swollen between the buccal aperture and the spiral organ, and below the endostyle, and appears missing in front of the siphons.

The V-shaped buccal siphon is narrow and protruding; it bears 20 smooth lobes. The rather wide muscle I is the sphincter; the one-fiber thick muscle II is a little behind it. The lateral muscle III, with about 15 fibers, is sinuous and S-shaped. An endodermal fold runs along this muscle and protrudes into the pharyngeal cavity. Muscles IV and V are present and close to the atrial siphon. This siphon has 20 long languets with a basal knot of epidermis and an apical tip of tunic. The dorsal process, found in *Doliopsis rubescens* lying above the atrial aperture (Kowalevsky and Barrois, 1883), was never observed in any specimen of this collection.

The nervous system only includes the neural ganglion (brain) and its nerves: a medio-anterior nerve, two pairs of latero-anterior nerves, a pair of lateral nerves, a pair of latero-posterior nerves (dividing early), and a pair of thin posterior nerves. A fine cellular thread (the former neural tube) joins the ganglion to the pharynx at the level of the ciliated funnel united with the locally broadened left peripharyngeal band.

The endostyle is a thin curved strip composed (Compère and Godeaux, 1997) of a medio-ventral ciliary band 1 (2 cells broad), a latero-ventral zone 2 with five to seven large glandular cells with a cell 2' at the top, and a lateral pseudostratified ciliated zone 3 (five to six nuclei on two ranks). Above, a less colored triangular zone with a single nucleus possibly corresponds to the zone 4 of the ascidian endostyle (Godeaux and Firket, 1968; Godeaux, 1981). A dense nucleus seems to correspond to the bridge joining the endostyle groove to the pharyngeal epithelium. The glandular bands form a cul-de-sac at both ends of the endostyle. The ciliary bands give off the peripharyngeal bands, which pass along muscle II, and unite in the apical spiral organ. This volute is provided with a finger-like process protruding into the pharynx. In photographs there is a cloud of scattered white pigment dots below the endostyle. The slightly obliquely positioned cardiopericardium lies behind the endostyle (Figs. 3B,D).

Up to 90 slits are present on both sides of the branchial septum. Their structure is similar to that of species of *Doliolum*.

The thin, vertical and almost linear esophagus is nearly as long as the rest of the digestive tract. It opens at the mid-height of the pharynx. Its main feature is the presence of three whorls running from its entrance down to the stomach. The globular and rather small stomach pouch is covered with a white-yellowish pigment, with some red pigment near the base. The pyloric gland emerges from the short duodenum; its tube obliquely runs towards the base of the ascending intestine and divides into two or three short branches, which ramify as thin cellular tubules over the distal intestinal wall (electron microscope observations, Compère and Godeaux, unpubl.). Numerous mesoblastic cells envelop the

descending intestine, duodenum included, and even the lower part of the stomach. These cells are pigmented white in the photographs, and their function is unknown. The ascending limb is naked. Fecal pellets are present in the intestine. The anal part is trumpet-shaped and opens into the atrial cavity.

In photographs, a whitish pigment is also present in the surface of the skin and of the branchial basket. The star-like red dot in front of the branchial basket and the red pigment dots above the siphons, observed by Kowalevsky and Barrois (1883) in *Doliopsis (Anchinia) rubescens*, are completely lacking.

The fixation stalk, visible below the rear of the endostyle and below the digestive tract, is completely embedded into the tunic, except for the naked adhesive disk, which is withdrawn into a deep hole of the tunic. Mature specimens were photographed attached to the same strip, creating a chain of blastozooids. The present position of the stalk is secondary and is explained by allometries of growth.

The species is hermaphroditic. The genital anlage in the younger specimens can be observed right at the bend of the gut. It is composed of a rather dense and rounded knot (a forming oocyte?) below a kind of triangle (the ovary?) and of a lateral elongate cluster of cells provided with a canal, the future testicular caeca and their spermiduct. Ripe gonads lie close to the gut. Depending on the specimen, one to five testicular caeca can be observed along the descending intestine (Figs. 5D,E), fusing into a thin vas deferens which runs along the bend of the gut, approaching the ventral ovary. The vas deferens opens into the lower part of the atrial cavity through a small funnel close to the oviduct. An oocyte is already present when two testicular caeca are visible. The maximum diameter observed for an oocyte in our collection was 400 mm. Possibly the ripe oocyte attains a greater size before it is shed. The oocyte is missing in older animals that have five caeca. Thus, the species appears protogynous.

Discussion.—This species differs from *Doliopsis rubescens* in its pigmentation, in the presence of three esophageal whorls, in the presence of an endodermal fold along muscle III, in the absence of the dorsal cloacal process and in the shape of the testis.

Paradoliopsis harbisoni Godeaux, 1996

Two collections were made at two widely separated locations, (station 2145, near George's Bank and station 1454, near the Dry Tortugas), at similar depths with almost the same ambient temperatures (Table 2). The specimens (Figs. 4,5F,6G,H) have similarities to members of the genus *Doliolum*, but also resemble members of the genus *Doliopsis*.

The first specimen (station 2145) is 2.2 cm long and 1.9 cm high when viewed from the side. It has a developing bud still attached to a ventro-posterior stalk (Figs. 4A,6G). An unattached young specimen also was present in the sample jar; it is attached to the zooid in the photograph.

The second specimen (station 1454) is 2.5 cm long and 2.3 cm high (Fig. 6H). Two unattached and identifiable buds also were present in the sample jar.

All six specimens displayed the same general structure.

The tunic of the first adult specimen is relatively thick, especially above the buccal siphon; it adheres loosely to the thin underlying epidermis.

The buccal siphon opens broadly (1.4 cm high) and has 24 lobes, one of which is medio-dorsal and one of which is medio-ventral.

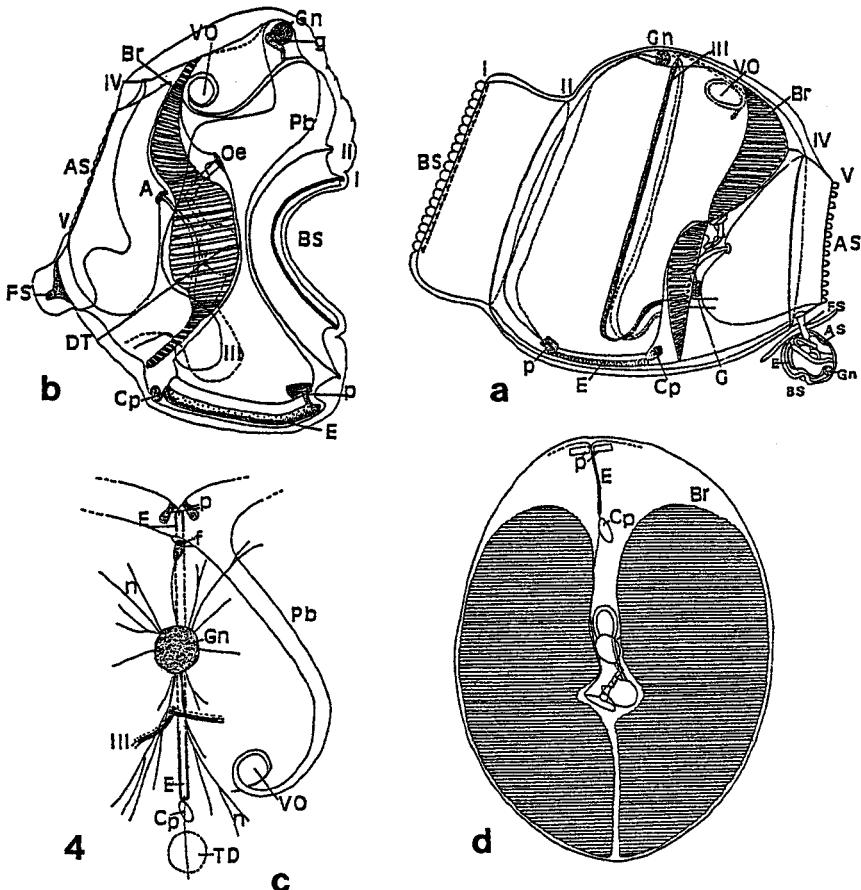


Figure 4. *Paradollopis harbisoni*. A) Full-grown gonozooid; B) Detached developing bud; C) Apical view of the full-grown gonozooid showing the neural ganglion, its nerves, and the location of the vibratile spiral organ; D) Frontal view of the branchial septum.

Five muscles are visible: Muscle I forms a ring immediately behind the buccal aperture, and is followed by a cylindrical vestibule (500 μm -long) delimited at the rear by muscle II, which forms a complete ring, the peripharyngeal bands and the pharynx.

Muscles III are composed of two long, non-united and symmetrical elements. Dorsally, the two parts overlap (they are contiguous in the second adult specimen) behind the neural ganglion. Each part extends vertically downward along the lateral wall of the pharynx toward the middle part of the endostyle and then bends backward to form a hook that ends behind the branchial basket. The endodermal epithelium is folded along these muscles, as observed in *Dollopis bahamensis* (see above), creating a kind of internal crest. The role of these folds remains unclear, but they may provide support for the pharyngeal wall. Muscle IV lies behind the gill; it is open ventrally directly in front of the stalk that carries the bud. Muscle V forms the atrial sphincter.

The nervous system is reduced to a dorsal ganglion (brain) and its nerves (Fig. 4C). As usual, a thin cellular thread links the ganglion to the ciliated funnel, which is in close

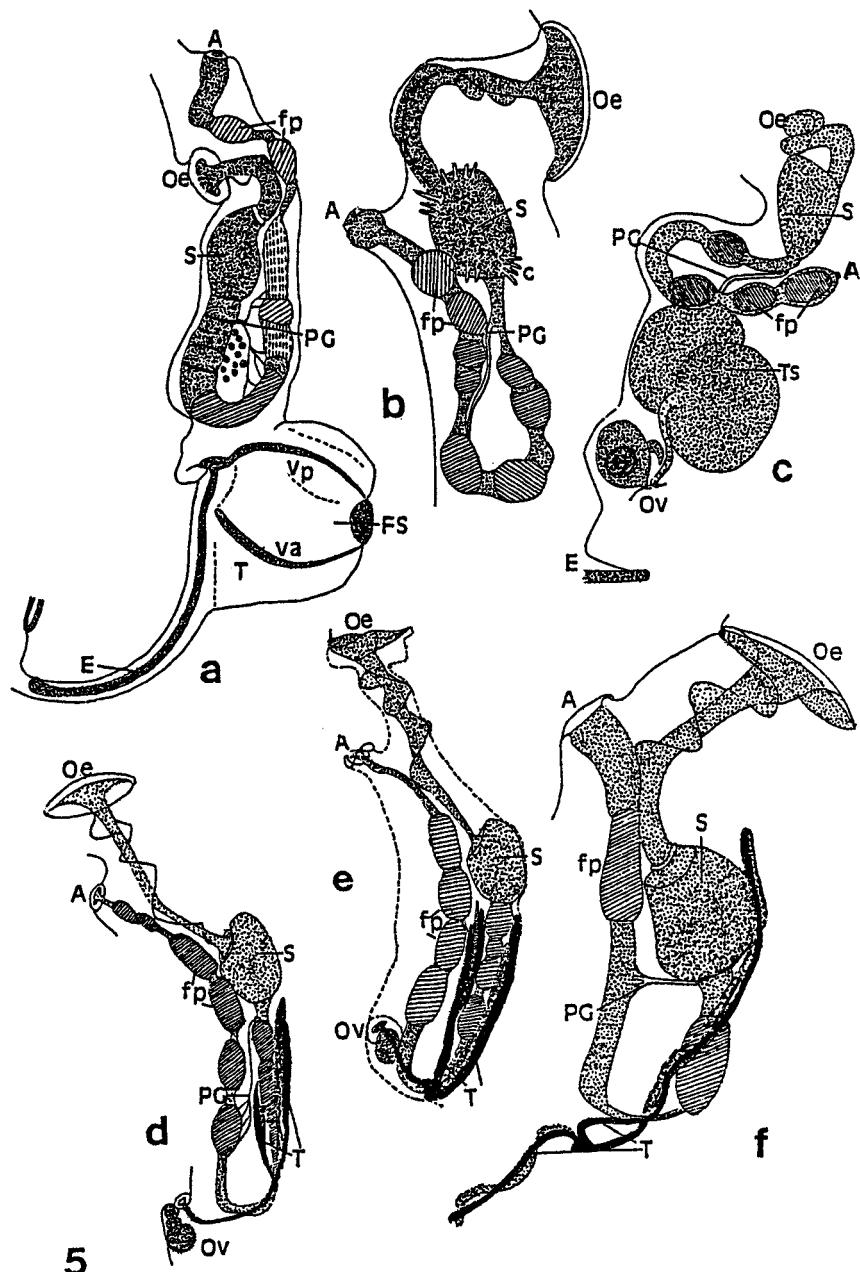


Figure 5. Digestive tracts and gonads of the different species. A) Digestive tract and ventral stalk of the full-grown trophozooid of *Doliolinetta intermedia*; B) Digestive tract of *Doliopsoides atlanticum*; C) Digestive tract and gonads of *Doliopsoides horizonti*; D,E) Digestive tract and testicular caeca of the gonozooids of *Doliopsis bahamensis*; F) Digestive tract with the four testicular caeca of a gonozooid of *Paradoliopsis harbisoni*.

contact with the left peripharyngeal band. There are numerous nerves. In front, two pairs of nerves supply the buccal lobes and muscles I and II; the size of their roots varies, as the nerves divide somewhat early. Two very thin nerves extend to the higher and the lower segments of muscle III. At the rear, two nerves with two lateral thinner nerves pass over muscles III and divide and further divide backwards (Fig. 5C).

The pharyngeal cavity is spacious, higher (1.5 cm) than longer (0.9 mm). Its ventral wall contains the endostyle (800 μm), underlain by a cloud of whitish pigment dots. At the front of the groove, two roughly rectangular clusters of cells filled with white pigment and some local yellowish-red blobs border the base of the peripharyngeal bands. The pigment is destroyed by preservation, leaving the cells colorless. Electron microscope views of cross sections of the endostyle resemble those of *Doliopsis bahamensis* (Compère and Godeaux, 1997). On both sides of the mid-ventral broad ciliated epithelium, two symmetric zones are visible, each composed of a lower cluster of about twelve large glandular cells and a pseudo-stratified epithelium containing four rows of dense elongated nuclei.

Two peripharyngeal bands run upwards around the buccal vestibule, pass *on the right side of the ganglion*, and unite behind muscles III into the spiral organ. It is noteworthy that the spiral organ is located on the *right* side of the animal, in front of the branchial wall, a position quite unusual in the doliolids (Fig. 4C). The retropharyngeal raphe and the vesicle-like cardio-pericardium are behind the endostyle.

The posterior wall of the pharynx is limited by the two sets of gill slits with a narrow epithelial zone in between (Fig. 4D). Each gill contains more than 60 transverse stigmata. The gill is vertical but is twisted and divided in two parts (Fig. 4A). The lower edge of each series has a white line extending into the esophagus.

The digestive canal appears as a wide U-shaped loop slightly inclined forwards. In photographs, the esophagus is pigmented red-orange from its funnel to the stomach, making the two whorls spiraling on the organ obvious. The ring of the esophageal opening is yellow-gold, as are the stomach and the intestine down to its bend. The rest of the intestine is colorless. All pigmentation is destroyed by fixation. The pyloric gland emerges below the stomach and divides into three branches in front of the ascending limb of the intestine. Probably these branches give off tubules, which run upward over this limb as has been observed in *Doliopsis bahamensis* (Compère and Godeaux, unpubl.). This part seems narrower and fecal pellets are absent. The anus opens at the tip of a small tubercle, at the level of the esophageal flexure.

The atrial cavity, behind the branchial septum, is rather wide, since muscle IV (the inner atrial sphincter) is separated from muscle V (the outer atrial sphincter) by a short distance. The atrial siphon has twenty-four isolated lobes of tunic.

The poorly developed gonad is composed of a 1 mm diameter mass located below the intestine. It has, on one side, a rounded oocyte close to the ovary, and has, on the other side, four tubular anlagen of the future testicular caeca, which are connected through a small pore with the atrial cavity. Protogyny seems probable.

The specimen bears a small blastozooid (1 \times 1 mm) ventrally at its rear (Figs. 4A, 6G). The yellowish stalk is located below the atrial cavity and behind the free ends of muscle IV. It appears as a protuberance of the haemocoelic cavity and does not resemble a true stolon such as is found in the oozoid of a species of *Doliolum*. Two clusters of cells are visible, which may represent future buds. The small blastozooid is well developed and its main organs (endostyle, peripharyngeal bands, spiral organ on the right side, neural com-

plex, and digestive loop) can be identified. The buccal siphon is distal, and the atrial siphon points slightly upward above the stalk, which is surrounded by a collar of tunic. In photographs, the digestive canal already appears yellowish-red. The neural gland, with its tube open into the pharynx and bound to the left peripharyngeal band, is conspicuous below the ganglion. The muscles (except possibly muscles III) were not observed with certainty. It appears that the buds develop successively on the process extending from the parent and are eventually shed.

Although very similar, the second perhaps older, adult specimen is a little larger, and exhibits some interesting differences from the previous specimen.

The testis is composed of four slender caeca, two stretching upwards along the digestive loop, two running downwards, uniting at the genital pore (Fig. 5F). No trace of the ovary is present.

Under the atrial siphon, this specimen has a whitish tubercle with a sticky distal area. On this tubercle a series of some twenty buds is present; the largest being 400 μm long, and is loosely attached, while the others are arranged in a S-shaped line, with two more developed (respectively 150 and 90 μm long) individuals located distally. All buds, except for the largest one, lie *beneath* the tunic.

The three unattached young free blastozooids ($5 \times 5 \text{ mm}$, $3.5 \times 3 \text{ mm}$ and $3 \times 3 \text{ mm}$ respectively) which became detached from the two large specimens confirm the above descriptions. They are simply small replicas of the adults (Fig. 4B). Possibly they are not yet active feeders, since their guts are empty. A thin and not yet thickened layer of tunic completely envelops the body. 30–45 stigmata are already open and more slits can be seen developing on both ends of the branchial septum. The endostyle already has the two anterior clusters of pigmented cells. Muscles are present. Muscles III are interrupted dorsally and already have longitudinal endodermal folds. The nervous complex consists of a dorsal ganglion and a ventral hollow neural gland; the canal of the gland opens into the pharynx through a funnel united with the left peripharyngeal band. This arrangement is quite similar to that observed in young blastozooids of species of *Doliolum*. A small cluster of cells, below and a little behind the gut, possibly represent the anlage of the future gonads.

On the fixation stalk below the atrial aperture, buds that are beginning to develop are seen (seven on the younger blastozooid). They are larger than the adjacent tunic cells and possibly multiply during the development of the carrier (e.g., the second adult specimen) as it is the case in gonozooids of other doliolids.

Discussion.—These animals have many features in common with members of the genus *Doliopsis*, but they are sufficiently different to justify (Godeaux, 1996) erecting the new genus *Paradoliopsis*, in the family Paradoliopsidae, with a single species, *Paradoliopsis harbisoni* (Table 3). The main differences are the presence of a buccal vestibule, the length and the position of muscles III, the position and the shape of the testicular caeca, the presence of two clusters of pigmented cells at the fore-end of the endostyle, the location of the vibratile spiral organ behind the neural ganglion *and on the right side of the body*, the presence of a ventral stalk bearing buds. Most of these characters are also visible in the young developing buds. The characters of the genus are those of the sole known species.

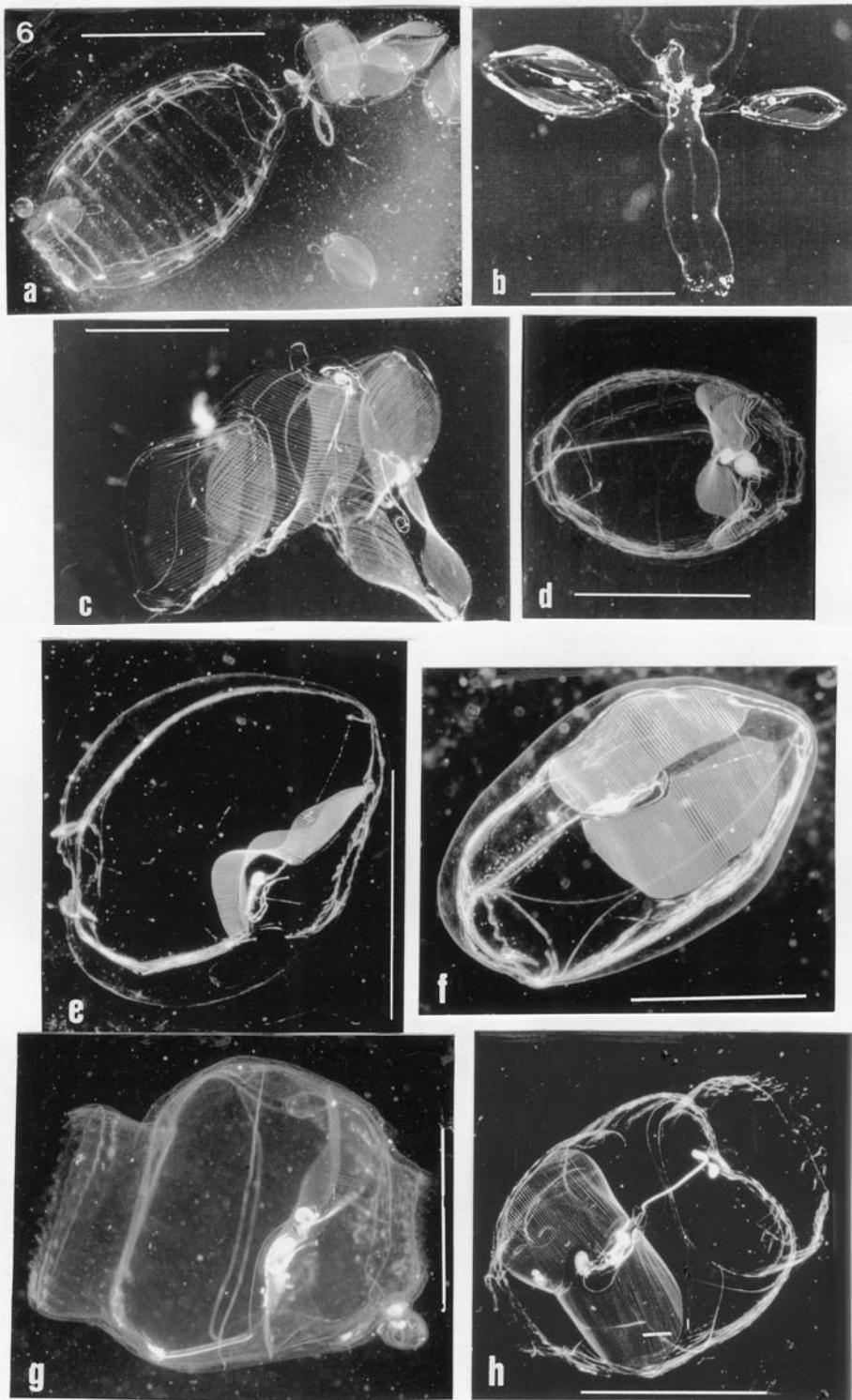
Remarks.—At station 2145 four young gonozooids and a developing bud of *Doliopsis bahamensis* were collected together with the specimen of *Paradoliopsis harbisoni* and are probably contaminants that resulted from an earlier unsuccessful attempt at collec-

Table 3. Comparison of living specimens of *Doliopsis rubescens* described by Kowalevsky and Barrois (1883) and Barrois (1885) with *Paradoliopsis harbisoni*.

<i>Doliopsis rubescens</i>	<i>Paradoliopsis harbisoni</i>
Body higher than longer	Body slightly longer than high
Tunic thick, particularly below the endostyle, scattered cells present	Tunic thick, especially above the buccal siphon
Buccal siphon narrow (10% of height)	Buccal siphon wide (75% of height)
Buccal vestibule short, muscles I and II close to one another	Buccal vestibule capacious, muscles I and II widely separated
Spiral organ lies dorsally behind the ganglion	Spiral organ shifted to the right side of the body, behind the ganglion and the muscles III
Endostyle short, with no pigment below it	Endostyle with two clusters of white pigmented cells at its front, and a cloud of white pigment lying under the endostyle
Endostyle reduced (<i>D.bahamensis</i>)	Endostyle reduced
Large spots of red pigment in front of the branchial basket and above the siphons	No red spots present
Stomach colorless	Red orange and yellow-gold pigments color the digestive system
White pigment on the lower gills	Lower gills colorless
Muscles III lateral, very short and S-shaped	Muscles III very long, overlapping dorsally and bent into an S-shape ventrally
Endodermal fold absent (present along muscles III in <i>D. bahamensis</i>)	Endodermal fold present running along muscles III
Muscle IV forms a complete ring	Muscle IV open ventrally
Atrial siphon short, with a slender process above it	Atrial siphon long, muscles IV and V separated, no dorsal process
No stolon, but an adhesive stalk	No stolon, but behind muscle IV, a stalk with developing buds embedded in the tunic
Trophozoid and gonozoid known	Gonozoid known
Short testicular caeca along the descending intestine	Four long, slender testicular caeca arranged in two opposed groups
A fixation stalk located below the endostyle	A posterior fixation stalk
Branchial slits arranged in a single row	Branchial slits missing at mid-height

tion. The hypothesis that the specimens of *Doliopsis* and of *Paradoliopsis* collected at the same station are simply different stages of the life cycle of the same species does not seem plausible, since there are significant differences in their anatomy (Table 3).

The life cycle of *Paradoliopsis harbisoni* is a matter of speculation. The animals described above are capable of sexual reproduction, but they also appear to be the carriers of buds produced by an as yet unknown individual, perhaps an oozooid. Buds develop into animals similar to the foster carrier. The egg may produce a type of individual able to bud off both the future gonozoids and the buds carried by the latter. Such a situation is known from the gonophorozoid of *Doliolum nationalis* (Braconnot and Casanova, 1967). The posterior stalk would then represent the attachment organ of the gonozoid to the oozooid, if one assumes that a phorozoid stage is missing.



GEOGRAPHIC DISTRIBUTION OF THE COLLECTED DOLIOLIDIDS

Doliolids were very rarely encountered: of 164 dives made with the JOHNSON-SEA-LINK submersibles from 1984–1991, doliolids were collected at only 16 stations. Quite remarkably, at four stations, more than one species was collected (Table 2). *Doliopsis bahamensis* co-occurred with each of the other three species, and was the only species to be found in all three areas (George's Bank, The Bahamas, and the Dry Tortugas). *Doliopsoides atlanticum* was collected only in the Bahamas, although the collections are too limited to draw any conclusions. *Paradoliopsis harbisoni* was collected at two widely separated stations (George's Bank and the Dry Tortugas), showing that it is widely distributed. Doliolids were never abundant at any of the stations where they were collected, although several specimens were observed but not collected at stations 1454 and 2145. Part of the problem with the identification of doliolids from a submersible is that they can easily be mistaken for salps or siphonophores, especially by untrained observers. Nevertheless, our collections suggest that doliolids are rare in the midwater regions of the North Atlantic where we made our dives, in marked contrast with their great abundance in shallower regions (Deibel, 1998).

SYSTEMATICS OF THE DOLIOLIDA

The collection described in this paper contains representatives (mainly as gonozooids) of four different genera of Doliolida, all belonging to different families. Godeaux (1996) suggested that the systematics of the order Doliolida needs to be revised, because of recent discoveries. Garstang's classic memoir of 1933 deals only with the family Doliolidae. The systematics of this family is based on the comparative structures of the phorozoids and of the gonozooids, for reasons defined by the author and are still valid. But other Doliolid species, such as *Doliopsis rubescens* (*Anchinia rubra*) (Kowalevsky and Barrois, 1883; Barrois, 1885) were not considered by Garstang.

All of the members of the different families of the order Doliolida have the following characters in common: (1) a barrel-shaped body with a thin ectoderm covered by a more

Figure 6. (opposite page) Photographs of living specimens taken shortly after collection. All scale lines, unless otherwise indicated, 1 cm. A) Lateral view of the nurse of *Doliolinetta intermedia* (station 1458-2) with a few adult trophozooids that became detached. B) View of the dorsal spur of the same nurse, bearing two developing trophozooids. Scale line = 0.5 cm. C) A group of four full-grown trophozooids detached from the same nurse. Scale line = 0.5 cm. D) Ventral view of a gonozooid of *Doliopsoides atlanticum* (station 1004-7). Scale line = 0.5 cm. E) View of the left side of a phorozoid of *Doliopsis bahamensis* (station 1681-2) showing the buccal and atrial siphons, the endostyle, the peribranchial bands, the apical vibratile organ, the U-shaped digestive tract, and the numerous gill slits of the branchial septum. F) Oblique dorsal view of a phorozoid of *Doliopsis bahamensis* (station 1681-2) showing the buccal siphon on the left, the ventral endostyle with a cloud of pigment cells around it, the origin of the peripharyngeal band running from the endostyle towards the apical vibratile organ, a partial oblique view of the two parts of the branchial septum that encircle the digestive tract, and the apical organ. G) View of the left side of a gonophorozoid of *Paradoliopsis harbisoni* (station 2145-8) showing the buccal and atrial siphons at opposite ends of the longitudinal axis, the ventrally-located endostyle with its two clusters of pigment cells, the dorsally-located brain in front of muscle III, the vertically-oriented U-shaped digestive tract and branchial septum between muscles III and IV, and a developed bud below the atrial siphon. H) Ventral view of the second gonophorozoid (station 1454-7), showing the buccal siphon on the right with its lobes, the ventrally-located endostyle with its two clusters of pigment cells, the branchial septum and the digestive tract, the atrial siphon on the left, and the peduncle of the bud.

or less thick tunic; (2) siphons open at both ends of the main axis; several (5 or 8) muscle bands encircling the body (which may be complete or not); (3) a single dorsal neural ganglion (brain); (4) an interior cavity divided into two parts, an anterior spacious pharyngeal cavity and a posterior narrow cloacal cavity, separated by a transverse branchial septum, the extent of which depends on the number of stigmata (from five to tens of slits); (5) a series of horizontal stigmata on both sides of the pharyngeal funnel, wherein each series of stigmata results from repeated divisions of a single embryonic dorso-ventral protostigma; (6) an endostyle lying in the ventral wall of the pharynx; (7) two peripharyngeal bands arising from the endostyle, encircling the buccal siphon and fusing dorsally into a volute; (8) the apical organ; (9) a cardiopericardium close to the rear of the endostyle; and (10) a very reduced abdomen which lies behind the branchial basket and only contains the digestive tract and the anlagen of the gonads.

The genera of the numerous species of Doliodidae can be distinguished by the shape of the digestive tract, while the species can be distinguished by the length of the endostyle, the size of the branchial basket and the number of gill slits, and, only in gonozoids, by the shape and the position of the testis.

Since the publication of Garstang's memoir, two new genera and several species have been described: namely, the genus *Doliopsoides* with its type species *Doliopsoides meteori* (Krüger, 1939); several other species of *Doliopsoides*; and the genus *Paradoliopsis* with the type species *Paradoliopsis harbisoni* (Godeaux, 1996). Furthermore, several species belonging to the genus *Doliolina* also were described (Tokioka and Berner, 1958a,b).

The revision proposed by Godeaux (1996) divides the order Doliodida into two suborders based on the number of muscle bands and the position of the vibratile spiral organ in gonozoids (Table 4):

- 1) The suborder Doliodida containing two families. Their representatives have eight muscle bands and the vibratile spiral organ lying in front of the neural ganglion.
- 2) The suborder Doliodina containing two families. Their representatives have five muscle bands and the vibratile spiral organ located behind the neural ganglion.

A dichotomous key defining the new suborders and the families is presented (Table 4).

Table 4. Dichotomous key to the suborders and families of Doliodida, based on the gonozoids.

- 1.— Body with eight muscle bands, vibratile spiral organ in front of the neural ganglion, shape of the gut and position of the testis variable.....Suborder Doliodida (2)
 - Body with five muscle bands, vibratile spiral organ behind the neural ganglion, U-shaped gut, testis near the gut.....Suborder Doliodina (3)
 - 2.— The eight muscle bands forming complete ringsFamily Doliodidae*
 - Muscles I, II, III, IV and VIII form complete rings, muscles V and VI open dorsally, forming arches, muscles VI and VII open ventrally and overlapping.....Family Doliodidae
 - 3.— Muscles I, II, IV and V form complete rings, muscle III short, S-shaped, lying on the flank. No ventro-posterior stalk bearing developing buds.....Family Doliodinae
 - Muscles I, II and V form complete rings, muscle III long, open ventrally, overlapping dorsally, with S-shaped ventral ends, muscle IV open ventrally, a ventro-posterior stalk bearing buds (gonophorozoids).....Family Paradoliopsisidae

* For more information on the systematics of the different genera and species in this family, see Godeaux (1998a).

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